EXHIBIT 37

DNA PROBES

Background • Applications • Procedures

SECOND EDITION

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Figure 6.6. After hybridization of the acridinium ester-labeled probe to its target nucleic acid, the acridinium moieties on the unhybridized probe molecules are selectively cleaved from the probe (and thus inactivated) by a proprietary process. The only chemiluminescence which remains is that associated with hybridized probe, so the remaining chemiluminescence is proportional to the amount of target nucleic acid. A drawback of the assay is detection sensitivity (about 1 ng of target), which makes this assay suitable only for the detection of amplified targets such as rRNA or PCR amplification products. Refer to Section 7 for further discussion of amplified targets.

SANDWICH HYBRIDIZATION

Solid Phase Sandwich Hybridization. The sandwich hybridization format was originally described by Dunn and Hassell (1977) and adapted by Ranki *et al.*

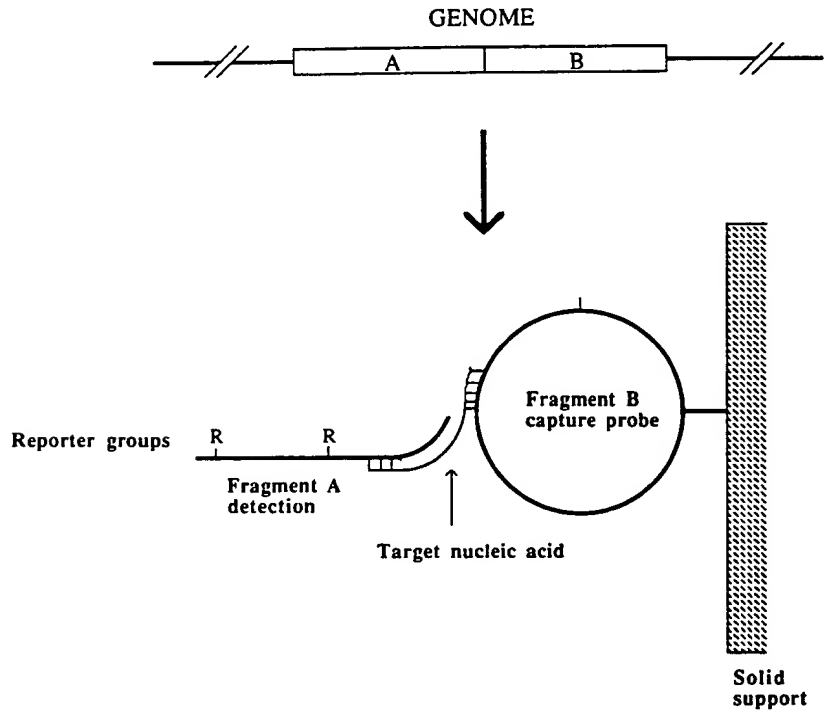

Figure 6.7 General Diagram of Sandwich Hybridization. Two adjacent DNA fragments from the genome of interest are cloned into non-homologous vectors. Here, the capture fragment (B) is cloned into M13 and immobilized, while the probe fragment (A) is cloned into pBR322, linearized and labeled. Probe is specifically bound to the support only in th presence of target nucleic acid that spans the junction between fragments A and B.

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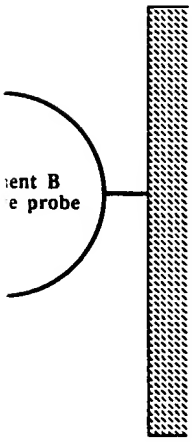

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(1983) and Ranki and Soderlund (1984). It was developed to avoid the tedious purification and immobilization of sample nucleic acid required in most solid phase hybridization formats. Sandwich hybridization has two main advantages over direct filter hybridization, sample immobilization is not required and crude samples can be assayed reliably. In addition, sandwich hybridization is potentially more specific than direct hybridization because two hybridization events must occur in order to generate a signal. Solid phase sandwich hybridization requires two adjacent, non-overlapping probes; an immobilized capture probe and a labeled detection probe. Figure 6.7 illustrates a typical sandwich hybridization scheme consisting of an immobilized capture sequence cloned into M13 and an adjacent detection sequence cloned into pBR322. A sandwich structure can form only if the sample contains nucleic acid which spans the original junction between the two fragments in genomic nucleic acid. Note that the two probes must be subcloned into separate, non-homologous vectors to avoid high background signals. Gel purification of the two adjacent fragments from the same clone is not suitable because, regardless of the care taken, each band will be contaminated with DNA from the other band.

Sandwich hybridization formats have utilized filters (Ranki *et al.*, 1983) and also utilized beads (Polsky-Cynkin *et al.*, 1985; Langdale and Malcolm, 1985) to immobilize the capture probe. The use of beads resulted in better standardization of the assays and easier handling of small numbers of samples. For large numbers of samples, however, beads can be difficult to handle and wash. Dahlen *et al.* (1987) have conducted sandwich hybridization in microtiter wells, which are more appropriate for handling large numbers of samples. They absorbed the capture DNA to the well, then fixed it to the plastic using UV light. Keller *et al.* (1989) used sandwich hybridization in microtiter wells to detect amplified nucleic acid fragments with a covalently coupled capture probe.

Sandwich hybridization in microtiter wells has a number of advantages over other hybridization formats. The use of sandwich hybridization provides specific signals using aliquots of the polymerase chain reaction (PCR) directly, even when the reaction contains crude cell lysate. This specificity is partly a result of the sample being *soluble* during the hybridization rather than being immobilized as with direct filter hybridization. This specificity is also due to the use of *two probes*, because two hybridization events must occur in order to generate a signal. The sensitivity of PCR product detection, using a photobiotinylated probe, is equivalent to Southern blotting with a ^{32}P-labeled probe (3×10^8 cpm/μg) and a 16 hour autoradiography exposure. Other advantages of using microtiter wells include the handling of 8-96 samples at a time, as well as multiple blocks of 96 samples, easy quantitation of the results and potential automation of the pipetting, washing and reading steps.